Effect of Bovine Somatotropin Administered to Periparturient Dairy Cows on the Incidence of Metabolic Disease

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ABSTRACT

Thirty-eight dry, pregnant Jersey cows were assigned to diet and bST treatment in a 2 × 2 factorial design. During the dry period, half of the cows were fed a normal TMR (0.4% Ca; 0.3 to 0.4% P), and half of the cows were fed a high Ca TMR (1.5 to 1.6% Ca; 0.4 to 0.7% P). The high Ca diets were designed to induce milk fever and were relatively cationic (194 to 293 meq/kg) compared with the normal diets (−131 to 30 meq/kg). A standard dairy diet was fed to all cows postcalving. Cows received subcutaneous injections of either an oil-based excipient or 500 mg of bST in an oil-based excipient every 14 d from 28 d before expected calving until approximately 14 d postcalving. Peripartal bST treatment decreased the incidence of clinical mastitis, did not affect incidence of milk fever, and increased the duration, but not the incidence, of ketosis in mature Jersey cows. Blood data confirmed the clinical responses and indicated that treated cows mobilized more bone Ca than did controls, as was evidenced by increased hydroxyproline concentrations. Treatment with bST did not affect blood concentrations of 1,25-dihydroxyvitamin D, Ca, or Mg. High Ca diets increased the incidence of milk fever and downer cow syndrome compared with normal diets. The effect of bST on mastitis and milk production must be considered as preliminary given the small size of the study. Although bST treatment increased Ca mobilization, the effect was insufficient to prevent milk fever in this model.
(**Key words**: bovine somatotropin, milk fever, lactation, mastitis)

Abbreviation key: **1,25-$(OH)_2$D** = 1,25-dihydroxyvitamin D, **BUN** = blood urea N, **HC** = high Ca, **HOP** = hydroxyproline, **NC** = normal Ca, **PTH** = parathyroid hormone, **ST** = somatotropin.

INTRODUCTION

Periparturient paresis (milk fever) occurs in 5 to 10% of all adult dairy cows in the US, although the incidence in individual herds varies from 0 to 60% (24). A significant decrease in the incidence of paresis following bST treatment for one lactation has been demonstrated (6, 7), but the acute effects of bST on postpartum metabolic disorders of dairy cows are unknown. A prophylactic effect of bST on milk fever was postulated for dairy cows based on these results (6). Associated complications, such as mastitis, ketosis, retained placenta, and dystocia, may also be improved by positive bST effects on milk fever. We postulated that bST could limit milk fever by increasing bone turnover (39) and Ca turnover as demonstrated for lactating cows (12). Somatotropin (**ST**) also has been shown to increase blood concentrations of 1,25-dihydroxyvitamin D (**1,25-$(OH)_2$D**) and intestinal mass to sustain elevated intestinal Ca absorption in growing pigs (15). In addition, bST has a general immunoenhancing activity that could be beneficial to disease resistance. Lean et al. (23) also postulated a reduced risk of metabolic disorders associated with lipid mobilization in the postparturient period following a lactation during which cows were treated with bST. The objective of this study was to examine the therapeutic effect of formulated bST on paresis and other health disorders of the periparturient dairy cow.

MATERIALS AND METHODS

Treatments

Thirty-eight dry, multiparous Jersey cows were grouped into five blocks of eight or six cows, based on initial body weight. Cows within a block were ran-

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domly assigned to diet and bST treatment in a 2 × 2 factorial arrangement. Cows were fed a TMR (Table 1) that was formulated to meet or to exceed NRC (28) requirements for Ca. Cows fed normal Ca (**NC**) diets received a TMR containing 0.4% Ca and 0.3% P beginning at dry-off. Beginning at 21 d prior to expected calving, NC cows were fed a TMR containing 0.4% Ca and 0.4% P. The cows fed the high Ca (**HC**) diets were fed a TMR containing 1.5% Ca and 0.4% P beginning at dry-off and a TMR with 1.6% Ca and 0.7% P beginning 21 d prior to expected calving. The HC diets were relatively cationic, averaging 194 to 293 meq/kg of DM; the NC diets averaged −131 to 30 meq/kg of DM. A standard dairy diet was fed to all cows postcalving (Table 1). The HC TMR were similar to diets previously demonstrated to predispose cows to hypocalcemia and milk fever after calving (16). All diets contained corn silage, chopped alfalfa hay, beet pulp, whole cottonseed, and premixed protein, grain, and mineral supplements.

Cows also received injections of either 0 or 500 mg of zinc methionyl bST every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Sterile bST was prepared and formulated in an oil-based excipient as described previously (12). Controls received an equivalent volume of excipient. All injections were administered subcutaneously into the left or right ischiorectal fossa. Results of the Jersey cow study were confirmed in a follow-up study conducted at the same facility with identical management. In that study, 89 Holstein cows received either no bST or 500 mg of zinc methionyl bST in the same formulations and the same regimen described previously for the Jersey study. For the follow-up study using Holsteins, only the NC diets were fed.

General

At least 30 d prior to the dry period, pregnant, multiparous Jersey cows were assembled at the Monsanto dairy facility (Dardenne, MO) to allow cows to become acclimated to handling and dietary regimens. All cows were screened for tuberculosis, brucellosis, paratuberculosis, and leukosis virus antibody titers prior to purchase. Selection criteria for the Jersey study and the follow-up Holstein study included recent clinical health, IMI, and previous milk production >5000 kg. Milk samples were taken prior to dry-off and analyzed for IMI as described subsequently; cows with pathogen isolates, such as *Staphylococcus aureus*, were excluded from the study. Although IMI was not used as a statistical blocking factor in assignment of treatment, the distribution of cows with nonpathogen isolates at dry-off was balanced across treatment groups. Cows used in the follow-up Holstein study were part of an ongoing treatment program, which included the same selection criteria. The exception was that the selected Holstein cows were not balanced by IMI but had pathogen-free milk isolates at dry-off. All cows received a commercial dry cow treatment at dry-off. Approximately 60% of the Jersey cows had a history of milk fever. Multiparous Jersey cows normally are more susceptible to milk fever (24) than are Holstein cows; the Jersey cows were fed HC diets during the dry period to exacerbate that tendency. This Jersey model previously has been demonstrated to elicit a 90% occurrence of paresis (18).

Cows were housed in an artificially lighted and ventilated tie-stall barn during both the dry and lactation periods. Two cows (1 control and 1 bST-treated cow fed the NC diet) were removed from study approximately 1 wk prepartum because of their failure to adjust to tie-stall management or handling. Data from all cows were retained in the health analysis, but cows must have completed at least two-thirds (6 wk) of the lactation period to be included in the production analysis. Ambient temperatures and humidity were measured daily at 0700 and 1600 h. Body weights were measured once weekly after the a.m. milking. Fresh feed was offered twice daily, and orts were measured prior to the a.m. feeding. Weekly feed samples were composited monthly and analyzed by Livestock Nutrition Lab Services (Columbia, MO). Calcium, P, and NE_L balance were calculated as previously described (12).

Cows were milked twice daily at about 0700 and 1900 h. At milking, teats were washed, dried with a paper towel, and forestripped. Following milking, all teats were dipped in an iodine solution. Weekly a.m. and p.m. milk samples were analyzed for fat and protein (Multispec infrared analyzer; Berwind Instrument Group, York, England), lactose (Milk-O-Scan 104 A/B; Foss Food Technology, Eden Prairie, MN), and SCC (Coulter milk cell counter; Coulter Electronics, Hialeah, FL).

Health

Daily clinical health observations were made during the study (dry-off until 63 ± 3 d after the subsequent calving). Guidelines of the National Institutes of Health (27) for the care and use of laboratory animals and the concepts embodied in the FDA *Good Laboratory Practice Guidelines* (19) were followed. All health-related observations were coded to indicate the literal clinical sign (e.g., estrus, milk fever, or

TABLE 1. Composition of the TMR fed to Jersey cows during the study.[1]

Nutrient content[2]	Dry cows				Lactating cows
	Normal Ca		High Ca		
	Diet 1	Diet 2	Diet 1	Diet 2	
NE_L, Mcal/kg	1.51	1.62	1.55	1.63	1.63
CP, %	12.3	13.8	19.0	17.6	17.1
ADF, %	32.9	26.3	30.6	25.5	25.1
Ca, %	0.44	0.39	1.49	1.59	0.91
P, %	0.25	0.43	0.35	0.65	0.47
Mg, %	0.16	0.25	0.29	0.29	0.29
K, %	0.79	0.92	1.80	1.47	1.07
Na, %	0.09	0.17	0.19	0.22	0.18
S, %	0.23	0.26	0.29	0.29	0.29
Cl^-, %	0.24	0.99	0.25	0.34	0.35
Fe, ppm	302	314	923	595	356
Zn, ppm	58	42	133	84	75
Mn, ppm	112	87	175	98	92
Cu, ppm	13	9	33	16	15
Cation-anion balance, meq/kg	30	−131	293	194	73

[1]Dry cows were switched from diet 1 to diet 2 at 21 d prior to expected calving. All cows were switched to the same diet at calving.

[2]All values are presented on a DM basis. Cation-anion balance was calculated as (Na/23) + (K/39) − (Cl/35.5) − (S/16). Chloride content was calculated from NRC (28) tables.

displaced abomasum) and the applicable body part (e.g., uterus, hoof, or tail). Prior to analysis, coded data were assigned to physiological categories, subsystems, and systems (13). Category codes described one or more clinical health signs. Subsystem codes contained a grouping of clinical signs that described the pathology of an organ or organ system. System codes were used to describe a vital function of the body: circulatory, digestive, genito-urinary, musculoskeletal, respiratory, and integumentary systems as well as udder, eye, metabolic, and miscellaneous disorders. Number of days medicated was categorized separately for mastitis and nonmastitic conditions. These data were summed to produce a subsystem code for total days medicated.

Clinical mastitis was diagnosed according to the presence of flakes in the milk or a hot, turgid appearance of the mammary gland. A milk sample from clinically positive cows was collected for microbiologic culture to identify the causative agent. A new case of mastitis was defined when at least 21 d had elapsed between reports of clinical mastitis, when a different quarter was affected, or when a different pathogen was isolated (13). Following milking, the affected quarter was treated with a commercial antibiotic, and all teats were dipped with an iodine solution. In addition, duplicate quarter milk samples were collected at d 7 ± 3 and 21 ± 3 of lactation to assess IMI status. During the follow-up study using Holsteins, duplicate quarter milk samples were collected at d 7 ± 3, 35 ± 3, 63 ± 3, and 91 ± 3 of lactation to assess IMI status. Cultures were declared to be invalid if contaminated (more than two bacterial species present) or if duplicate sample results did not agree. For reporting purposes, isolates were grouped into the following categories: 1) *S. aureus*, 2) coagulase-negative staphylococci, 3) Gram-negative lactobacilli or coliforms, 4) streptococci, 5) and other (e.g., *Corynebacterium bovis*, *Actinomyces pyogenes*, and yeast).

All cows were dewormed at parturition. At 3 wk postpartum, cows received a commercial vaccine against bovine respiratory syncytial virus, infectious rhinotracheitis, viral diarrhea, and parainfluenza. All cows also received a vaccination against leptospirosis. If a cow was treated medically for milk fever or ketosis, a serum sample was drawn prior to treatment to confirm the diagnosis. For data analysis, cows exhibiting recumbent paresis and serum Ca <6 mg/dl were considered to have milk fever. Based on clinical symptoms, cows were treated intravenously with Ca gluconate (0.42 *M* Ca; 500 ml). Cows that exhibited inappetence and positive urinary ketones by reagent strip analysis (Chemstrip K; Boehringer Manheim Diagnostics, Indianapolis, IN) were considered to be ketotic. Ketosis was treated intravenously with 50% dextrose or, if unresponsive, with oral propylene glycol. The ketosis was commonly observed during inappetence at parturition or during treatment for displaced abomasum; primary ketosis was not observed. Beginning 35 d prior to expected calving, rectal temperatures were measured daily at 1500 to 1700 h. At 3 and 10 d after bST administration, injection sites were scored for swelling (0 = none, 1 = minimal, 2 = moderate, and 3 = severe).

Blood Analyses

Blood samples were collected daily from the Jersey cows by coccygeal venipuncture at approximately 1200 to 1300 h from 14 d prior to expected calving until 7 d postpartum. Serum and plasma were frozen for later analysis of hormones and clinical chemistries. Hydroxyproline (**HOP**) concentrations were measured by colorimetric procedure (9). Plasma 1,25-$(OH)_2D$ concentrations were determined by radioreceptor assay (29). Blood glucose, Ca, P, Mg, Na, K, Cl^-, total protein, BSA, creatinine, triglycerides, total bilirubin, and urea N (**BUN**) concentrations were analyzed using a Dimension clinical chemistry analyzer (model 380E; DuPont, Wilmington, DE). Serum NEFA concentrations were measured using an enzymatic assay (Wako Pure Chemical Industries Ltd., Richmond, VA). Blood ST, insulin, and IGF-I concentrations were measured by radioimmunoassay as previously described (11, 37). Parathyroid hormone (**PTH**) concentrations were measured using a commercial kit (Diagnostic Products Corp., Los Angeles, CA). The PTH kit was validated for bovine PTH by parallelism (comparative slope = 1.009) and recovery (70%). Similar recoveries were obtained previously for PTH using commercial kits (17). Intraassay and interassay coefficients of variation averaged 6.4 and 4.4% for PTH, 10.9 and 17.5% for ST, 10.4 and 14.0% for insulin, and 8.9 and 5.9% for IGF-I.

Urine Analyses

Three daily urine samples were collected from each Jersey cow at 1100 to 1500 h between approximately 7 and 4 d prior to calving. Urine pH was measured using a solution analyzer (model 4503A; Amber Science, San Diego, CA). A 1.5-ml subsample was then acidified with 15 ml of concentrated nitric acid (14) and frozen for analysis of creatinine using a commercial kit (creatinine number 555; Sigma Chemical Co., St. Louis, MO) and HOP (21).

Statistics

Statistical analyses were conducted using SAS (31). The logistic procedure of SAS was used to conduct logistic regression analysis on health data by category, subsystem, and system (13) using a model containing terms for the main effects of diet and treatment expressed as binary variables. Model terms were evaluated using Wald statistics (1). Because of small observed counts, logistic regression models containing the additional interaction term treatment by diet were nonestimable for most variables. Thus, health analyses were limited to the main effects model. Differences in the incidence of a health disorder were considered to be significant if $P < 0.1$ (13), which was selected for the clinical health and blood chemistry data based on expected coefficients of variation, study size constraints, and the protocols for previous FDA health analyses (13). Subclinical IMI data, expressed as a percentage positive or negative, were analyzed using a model that included the effects of diet and treatment.

Blood, urine, milk, and feed data were examined by ANOVA using the GLM procedure of SAS (31). Data were examined for normality and heterogeneity of variance (32) and, if necessary, transformed according to Box et al. (2). Blood data were analyzed by repeated measures ANOVA using a model that included diet, bST, day of lactation (−7 d through 4 or 7 d), all two- and three-way interactions, and cow within treatment group. The effects of diet, bST, and the interaction of diet and bST were tested with cow within treatment group as the error term. Other effects were tested with the model residual as the error term. Individual cow means for milk production and composition, feed intake, and urine data were analyzed using a model that included the effects of diet, bST, weight block, the interaction of bST and diet, and residual error. The ANOVA model in the follow-up study using Holsteins did not include diet because only the NC diet was fed in that trial. Differences in production data means were considered to be significant if $P < 0.05$.

RESULTS

Clinical health data for the Jersey cows are summarized in Table 2. Somatotropin treatment did not affect incidence of milk fever, but HC prepartal diets increased ($P = 0.05$) the incidence of milk fever (8 of 17 cows) and downer cow syndrome ($P = 0.04$; 7 of 17 cows) compared with those fed the NC diet (3 of 19 and 2 of 19 cows, respectively). Three cows (1 control cow fed the NC diet and 2 treated cows fed the HC diet) failed to respond to medical treatment and died from milk fever within 3 d of calving (data from all cows were retained in the health analysis). Neither bST nor diet affected ($P > 0.1$) the incidence of health disorders of the circulatory-lymphatic, genito-urinary, respiratory, optical, or integumentary systems or the incidence of elevated body temperature or allergic reaction. Swelling of the injection site was minimal to nonexistent and did not affect cow health (data not presented). With the exception of subclinical mastitis (discussed subsequently), the incidence of health disorders in the Holstein study was unaffected by bST treatment (data not presented).

In agreement with the data for milk fever, bST did not affect ($P > 0.1$) blood 1,25-$(OH)_2D$, Ca, or Mg concentrations in Jersey cows, which averaged 51.6 pg/ml, 8.8 mg/dl, and 2.2 mg/dl, respectively (Figure 1). The interaction of diet and bST was not significant ($P > 0.1$) for any blood analyte (data not presented). The minimum concentrations of 1,25-$(OH)_2D$, Ca, and Mg and the day on which they occurred also were not affected by bST: 122.8 pg/ml, 6.0 mg/dl, and 1.6 mg/dl on d 1, 1, and 3 of lactation, respectively. Compared with the NC groups, cows fed HC diets had higher ($P < 0.01$) overall blood Mg (2.3 vs. 2.1 mg/dl) and BUN ($P = 0.04$; 13 vs. 11 mg/dl). Cows fed HC diets also had lower overall blood Ca (8.6 vs. 9.0 mg/dl), 1,25-$(OH)_2D$ (45.3 vs. 58.0 pg/ml), and creatinine (0.97 vs. 1.06 mg/dl) concentrations than did cows fed NC diets ($P = 0.02$). These effects were most notable during the prepartum period when the HC diets were fed; the interaction of diet and day of study was significant ($P < 0.1$) for lower postpartum Mg concentrations for cows fed HC diets. Cows fed HC diets prepartum also had a higher ($P < 0.01$) overall urine pH (pH 8.0) than did cows fed NC diets (pH 6.0); bST treatment during the periparturient period did not affect urine pH ($P > 0.1$; Table 3). Urine creatinine concentration was increased ($P = 0.04$) in cows treated with bST, primarily those in the NC group. The interaction of diet and bST was not significant ($P > 0.1$) for urine pH or creatinine.

Day relative to parturition significantly affected all blood analyte concentrations except PTH. The timing of spikes in PTH concentration relative to parturition of individual cows was highly variable, and no effect

TABLE 2. Effect of a normal (NC) or high Ca (HC) diet[1] and treatment with bST (none or 500 mg/14 d) administered to periparturient Jersey cows on clinical health disorders observed from approximately 56 d prepartum until 63 d postpartum.

Clinical observation	Treatment: bST 0 mg	bST 500 mg	Diet NC	Diet HC	P: bST	P: Diet
Milk fever						
Cows, no./total no.	6/19	5/17	3/19	8/17	0.88	0.05
Total days observed	13	7	4	18	0.67	<0.01
Downer cow syndrome						
Cows, no./total no.	5/19	4/17	2/19	7/17	0.84	0.04
Total days observed	10	5	3	12	0.34	0.03
Off feed (prepartum)						
Cows, no./total no.	4/19	4/18	6/19	2/18	0.85	0.16
Total days observed	7	13	18	2	0.16	<0.01
Ketosis						
Cows, no./total no.	6/19	8/17	6/19	8/17	0.33	0.33
Total days observed	21	35	27	30	0.03	0.51
Diarrhea						
Cows, no./total no.	2/19	6/17	4/19	4/17	0.08	0.84
Total days observed	4	11	8	7	0.05	0.94
Days with clinical observation						
Prepartum						
Cows, no./total no.	9/19	7/17	11/19	5/17	0.69	0.09
Total days observed	21	24	31	14	0.43	<0.01
Postpartum						
Total days observed	89	95	81	103	0.19	0.04
Days medicated (nonmastitic cows)	91	102	87	106	0.09	0.07

[1]Diets contained 0.2 to 0.4% Ca (NC) or 1.2 to 1.5% Ca (HC) and were fed from dry-off until the subsequent calving. Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Postpartum observations are listed unless otherwise stated. Probability values are presented for the main effects of diets and bST treatment.

TABLE 3. Effect of a normal (NC) or high Ca (HC) diet[1] and treatment with bST (none or 500 mg/14 d) administered to periparturient Jersey cows on prepartum urine pH, hydroxyproline (HOP), creatinine (CREA), and HOP:CREA.

Variable	Treatment								P		
	bST				Diet						
	0 mg		500 mg		NC		HC		bST	Diet	bST × Diet
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE			
pH	6.9	0.12	7.0	0.12	5.9	0.12	8.0	0.12	0.40	<0.01	0.15
HOP, μg/ml	4.63	0.909	6.24	0.938	6.14	0.911	4.75	0.940	0.23	0.30	0.11
CREA, mg/dl	69.0	12.00	104.9	12.39	100.5	12.04	73.4	12.42	0.04	0.13	>0.10
HOP:CREA	8.1	1.63	9.3	1.69	7.3	1.64	10.1	1.69	0.59	0.24	0.89

[1]Diets contained 0.2 to 0.4% Ca (NC) or 1.2 to 1.5% Ca (HC) and were fed from dry-off until the subsequent calving. Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Urine samples were collected from approximately 7 to 4 d prior to parturition. Probability values are presented for the main effects of bST treatment, diets, and the interaction of bST treatment and diet.

was noted for bST or diet (Figure 1). However, the interaction of diet and day of lactation was significant because the mean PTH concentrations of untreated cows fed the NC diet did not peak after calving (Figure 1). Blood HOP concentrations ($P < 0.1$) were elevated in treated cows. Prepartal urine HOP concentrations were unaffected ($P > 0.1$) by bST but tended to be higher for treated cows fed the NC diet. The interaction of diet and bST treatment was nonsignificant ($P > 0.1$) for urine HOP (Table 3).

The overall number of cases of mastitis was marginally lower ($P = 0.1$) for Jersey cows treated with bST than for untreated cows (Table 4) because no mastitis was observed for treated cows fed the HC diet. There was no apparent effect of bST on mastitis for cows fed NC diets. The number of Jersey cows with IMI was unaffected by bST or diet ($P > 0.1$). The bacteriological status of clinically and subclinically mastitic cows generally supported the clinical trends (Table 4). In the follow-up study using Holsteins in which NC diets were fed, bST did not affect ($P > 0.1$) the incidence of clinical mastitis (Table 5). Fewer treated Holstein cows (28%) than control cows (38%) had subclinical infections ($P < 0.01$; Table 5). The total number of days that Jersey cows received a medication for mastitis was lower ($P < 0.01$) for cows fed the HC diets; treatment days were unaffected by bST treatment (Table 4). Conversely, both HC diets and bST increased the number of days that cows received medication for nonmastitis health disorders (Table 2). The total days that Jersey cows were observed with an udder disorder was increased for cows in the bST group because 2 cows fed the NC diet had teat injuries that were present for 9 d. One of those cows was removed from study because of the injury (data from all cows were retained in the health analysis).

Blood insulin, glucose, triglyceride, P, Na, K, Cl^-, BSA, total protein, and bilirubin concentrations of the Jersey cows were unaffected ($P > 0.1$) by bST and diet (data not presented). However, blood ST and IGF-I concentrations increased ($P < 0.1$) in cows treated with bST (Figure 2). Serum ST averaged 5.3 ng/ml for bST-treated cows and 2.0 ng/ml for untreated cows. Plasma IGF-I concentrations were elevated ($P < 0.1$) in bST-treated cows prior to parturition but decreased for all treatment groups at calving (Figure 2).

Diet did not affect ($P > 0.05$) 3.5% FCM or milk fat, protein, lactose, or somatic cell concentrations of milk from the Jersey cows through the first 9 wk of lactation (Table 6). Periparturient bST treatment did not affect milk composition but was associated with decreased ($P < 0.05$) FCM production, primarily in the NC group after approximately 4 to 5 wk of lactation (Figure 3). Overall production averaged 26.9 ± 1.25 kg/d for controls compared with 22.6 ± 1.28 kg/d for the cows treated with bST. The interaction of diet and bST was not significant ($P > 0.05$) for milk or milk composition. In the follow-up study, FCM of Holstein cows treated with bST during the periparturient period was unaffected by bST (39.0 ± 1.03 kg/d overall). Milk SCC in that study averaged 49,000 cells/ml and was not affected by treatment.

Neither feed DM nor NE_L intake of Jersey cows was affected ($P > 0.05$) by bST treatment or diet either prepartum (9.0 kg/d; 14.2 Mcal/d) or during the first 9 wk postpartum (13.3 kg/d; 22.8 Mcal/d). The interaction of diet and bST was not significant ($P > 0.05$) for intake parameters (Table 7). Although not significantly different, cows fed NC diets prepartum had slightly higher postpartal DMI (14.0 kg/d) than did cows fed the HC diet (12.7 kg/d). Similarly, mean energy balance tended ($P > 0.05$) to be greater for cows in the NC group (2.47 Mcal/d) than for cows in the HC group (1.09 Mcal/d). As expected, prepartum nutrient intakes differed according to the diets fed. For example, Ca intake during the 3 wk immediately prepartum averaged 153 g/d for cows fed the HC diets compared with 35 g/d for cows fed NC diets prepartum. Postpartum Ca intakes followed the same trend in DMI; cows fed NC diets had greater ($P < 0.05$) Ca intakes (146 g/d) than did cows fed HC diets (94 g/d). Calculated Ca, P, and protein balances postpartum were not affected by bST or prepartal diets (data not presented).

The number of days that Jersey cows were off feed prior to calving was increased for cows fed the HC diets. More treated cows ($P < 0.1$) had diarrhea than untreated cows (Table 2). For example, 6 cows that were treated with bST had diarrhea or loose stools for a total of 11 d (mean, <2 d each), and 2 untreated cows had a total of 4 d (mean, 2 d each). Although the number of cows observed with secondary ketosis was not affected by treatment ($P > 0.1$), the total number of days of ketosis was increased for cows treated with bST (Table 2). The blood ketone, BHBA, was increased (data not presented) to approximately 2.80 m*M* in cows treated with bST, supporting the observation of increased days with clinical ketosis.

DISCUSSION

The lack of a bST effect on the frequency of cases of milk fever confirmed previous work with cows fed pasture (22) in which slow-release bST also showed no effect on the incidence of milk fever or blood Ca concentrations in the periparturient period. Elevated blood Mg and lower blood Ca and 1,25-$(OH)_2D$ concentrations prepartum confirmed that the HC diets

TABLE 4. Effect of a normal (NC) or high Ca (HC) diet[1] and treatment with bST (none or 500 mg/14 d) administered to periparturient Jersey cows on mammary health and mastitis.

Clinical observation	Treatment: bST 0 mg	bST 500 mg	Diet NC	Diet HC	*P* bST	*P* Diet
Udder disorder (injury)						
Cows, no./total no.	3/19	4/17	4/19	3/17	0.56	0.79
Total days observed	3	11	10	4	0.03	0.17
Clinical mastitis						
Cows, no./total no.	7/17	5/16	8/17	4/16	0.46	0.17
Cases/1000 cow days[2]	24.0	9.5	17.4	16.1	0.10	0.95
Isolate, %[3]						
Staphylococcus aureus	67	25	50	60	NA[4]	
CNS	22	0	0	40	NA	
GNR	0	25	13	0	NA	
Streptococci	11	50	37	0	NA	
Other	0	0	0	0	NA	
Days medicated (mastitis)	45	32	51	26	0.20	<0.01
Subclinical IMI, % of cows						
Negative	25	50	58	17	0.20	0.12
Positive	75	50	42	83	0.20	0.12
Isolate, %						
S. aureus	14	0	0	13	NA	
CNS	36	86	33	60	NA	
GNR	0	0	0	0	NA	
Streptococci	21	14	67	0	NA	
Other	29	0	0	27	NA	

[1]Diets contained 0.2 to 0.4% Ca (NC) or 1.2 to 1.5% Ca (HC) and were fed from dry-off until the subsequent calving. Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Postpartum observations are listed unless otherwise stated. Probability values are presented for the main effects of diets and bST treatment.

[2]Mastitis excludes notations of atypical milk during the first week postpartum. One mastitis case per 63-d lactation period during the study = (1 case/63 d) = 0.016 cases/cow day = 16 cases/1000 cow days (13).

[3]CNS = Coagulase-negative staphylococci, GNR = Gram-negative bacteria or coliforms, and other = *Corynebacterium bovis*, *Actinomyces pyogenes*, and yeasts.

[4]Not analyzed.

fulfilled the study design and had a metabolic effect on the cows. The changes in BUN and urine and blood creatinine were not consistent with renal dysfunction; concentrations remained within normal ranges.

As shown in Figure 1, prepartal bST treatment did not uncouple the vitamin D and Ca axis in either dietary group. The lack of effect of bST or diet on PTH concentration or the timing of concentration spikes relative to parturition confirmed the results of earlier work (34). In other species, ST has been demonstrated to improve intestinal Ca absorption and stimulate renal 1-hydroxylase, resulting in increased blood 1,25-$(OH)_2D$ concentrations (34). Moreover, elevated blood HOP concentrations indicated that cows treated with bST mobilized more bone Ca than did controls, although the clinical incidence of milk fever was not improved. Somatotropin also has been shown to stimulate bone turnover in other species (39). Because most of the Ca that is available to the cow is of dietary origin and is regulated by vitamin D

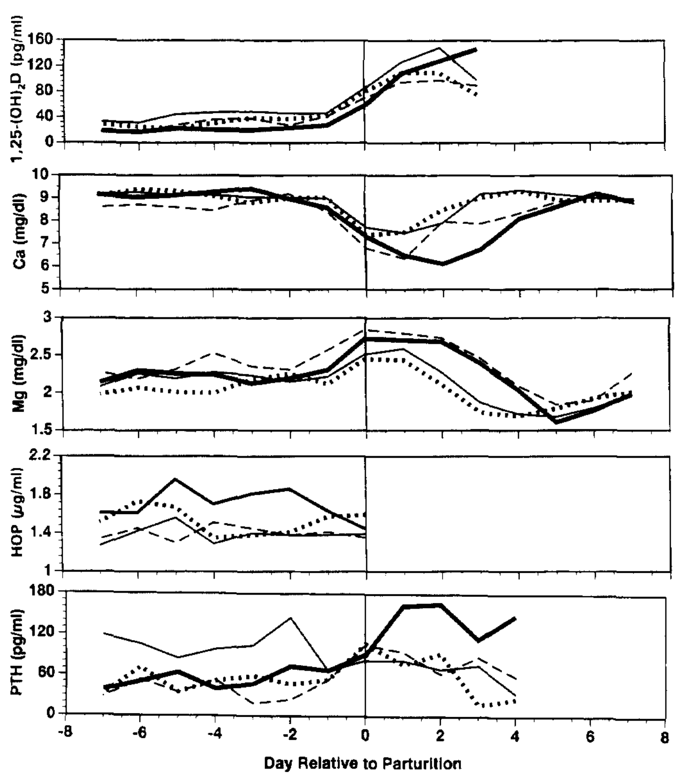

Figure 1. Effect of a normal (NC) or high Ca (HC) diet and excipient or 500 mg of bST in excipient/14 d on blood 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$), Ca, Mg, hydroxyproline (HOP), and parathyroid hormone (PTH) concentrations of cows during the periparturient period. Pooled standard error = 5.10 pg/ml, 0.16 mg/dl, 1.03 mg/dl, 0.08 g/ml, and 10.83 pg/ml for 1,25-$(OH)_2D$, Ca, Mg, HOP, and PTH, respectively. Treatments: excipient with the HC (dashed line) or NC (thin line) diet or 500 mg/14 d of bST with the HC (thick line) or NC (dotted line) diet.

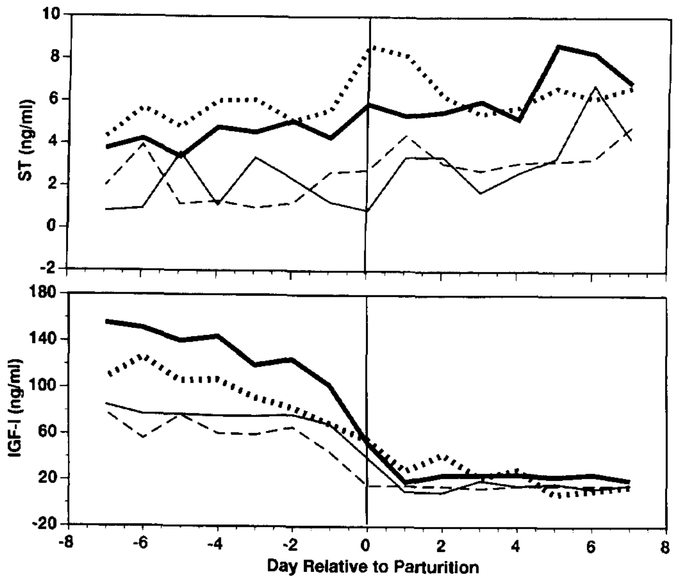

Figure 2. Effect of a normal (NC) or high Ca (HC) diet and excipient or 500 mg of bST in excipient/14 d on blood somatotropin (ST) and IGF-I concentrations of cows during the periparturient period. Pooled standard error = 1.21 and 10.83 ng/ml for ST and IGF-I, respectively. Treatments: 0 mg of bST with the HC (dashed line) or NC (thin line) diet or 500 mg/14 d of bST with the HC (thick line) or NC (dotted line) diet.

(30), the lack of effect on blood 1,25-$(OH)_2D$ concentration supports the absence of a clinical effect. In contrast, porcine ST increased blood 1,25-$(OH)_2D$ concentrations and intestinal mass to sustain elevated intestinal Ca absorption in growing pigs

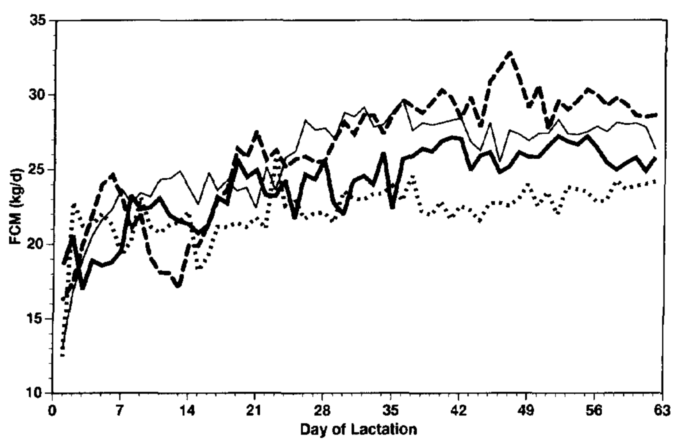

Figure 3. Effect of a normal (NC) or high Ca (HC) diet and excipient or 500 mg of bST in excipient/14 d on 3.5% FCM production from calving through 63 d of lactation. Pooled standard error = 1.77 kg/d. Treatments: excipient with the HC (dashed line) or NC (thin line) diet or 500 mg/14 d of bST with the HC (thick line) or NC (dotted line) diet.

(15). Although bST has been reported to increase the weight of the gastrointestinal tract of cows (3) and although treatment during established lactation increases Ca turnover (12), Ca absorption likely was not increased in our cows (not measured) as was evidenced by the lack of effect on the incidence of milk fever.

The lower overall number of cases of mastitis in bST-treated Jersey cows should be viewed as a possible trend with marginal statistical significance (P = 0.1) because improved mastitis was observed only for bST-treated cows fed the HC diet. There was no apparent effect of bST on mastitis in Jersey cows fed NC diets or on subclinical status overall. The proportion of pathogens present in clinical isolates and positive subclinical isolates was in general agreement with the profile of clinical mastitis (Table 4). The follow-up study using Holsteins showed a decrease in the number of cows with subclinical infections: 38% of the control cows versus 28% of the treated cows. Those

TABLE 5. Effect of treatment with bST[1] (none or 500 mg/14 d) administered to periparturient Holstein cows on mastitis and subclinical IMI.

Observation	bST 0 mg	bST 500 mg	P
Clinical mastitis			
Cows, no./total no.	10/47	11/42	0.61
Cases/1000 cow days[2]	5.4	4.6	0.83
Isolate, %[3]			
Staphylococcus aureus	58	20	NA[4]
CNS	0	20	NA
GNR	8	25	NA
Streptococci	33	35	NA
Other	0	5	NA
Subclinical IMI, % of cows			
Negative	62	72	0.01
Positive	38	28	0.01
Isolate, %			
S. aureus	22	9	NA
CNS	54	56	NA
GNR	2	5	NA
Streptococci	17	11	NA
Other	5	19	NA

[1]Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. All cows were fed a diet that contained 0.2 to 0.4% Ca (the NC diet in the Jersey study). Probability values are presented for the main effect of bST.

[2]Mastitis excludes notations of atypical milk during the first week postpartum. One mastitis case per 98-d lactation period during the study = (1 case/98 d) = 0.010 cases/cow day = 10 cases/1000 cow days (13).

[3]CNS = Coagulase-negative staphylococci, GNR = Gram-negative bacteria or coliforms, and other = *Corynebacterium bovis*, *Actinomyces pyogenes*, and yeasts.

[4]Not analyzed.

data are consistent with the observation that mitogen-induced lymphoblastogenesis was significantly potentiated in bST-treated Jersey cows fed HC diets (8). The relationship between HC diets, bST, leukocyte function, and mastitis deserves further study. Although many other components, including neutrophilic activity, are part of the response of cows to mastitis, bST may augment the ability of the cow to fight infection. Burvenich et al. (5) reported a more rapid return to normal milk production following acute mastitis for bST-treated cows than for controls. However, during established lactation, exogenous bST either has not affected (10) or moderately increased (4, 6, 13) the incidence of clinical mastitis. Daily injection of 25 mg of bST/d from d −21 to −7 relative to expected calving (38) did not affect linear SCS for treated cows (3.0 ± 0.1) over those of controls (3.4 ± 0.1).

Blood ST and IGF-I concentrations were increased in cows treated with bST prior to calving, but IGF-I concentrations decreased for all treatment groups at calving (Figure 2). Similarly, cows treated with 5 or 14 mg of bST/d during the dry period had elevated ST and IGF-I concentrations during the first 23 d of treatment (20, 33), but IGF-I concentrations were decreased as the date of calving approached. In addition, concentrations of IGF-binding protein-3 and IGF-binding protein-2 decreased during the peripartum period for cows treated with bST (33). Simmons et al. (33) postulated that negative energy balance was associated with the periparturient changes in IGF-I and IGF-binding protein response. Perhaps in light of the decrease in IGF-I concentrations at calving, a positive effect of bST on milk fever was unlikely to occur. Bone resorption is directly affected by IGF-I stimulation of Ca release (34).

Although prepartum HC diets increased the incidence of milk fever, response to medical treatment was apparently not compromised because 3.5% FCM production was unaffected by diet through the first 9 wk of lactation (Table 6). The cause of the decrease in milk production of bST-treated Jersey cows was uncertain but might have been related to the increased incidence of diarrhea and ketosis of that group. The FCM of Holstein cows that were treated during the periparturient period in the follow-up study was unaffected by bST treatment, confirming earlier reports (35, 38). Daily injection of 25 mg of bST/d from d −21 to −7 precalving also did not affect FCM production (38). Stelwagen et al. (35) have previously reported a decrease in early lactation milk production in heifers treated with 40 mg of bST/d during the last trimester of gestation. However, heifers treated with 20 mg of bST/d prepartum had

TABLE 6. Effect of a normal (NC) or high Ca (HC) diet[1] and treatment with bST (none or 500 mg/14 d) administered to periparturient Jersey cows on milk production and composition through 9 wk of lactation.

	Treatment								P		
	bST				Diet						
Variable	0 mg		500 mg		NC		HC		bST	Diet	bST × Diet
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE			
3.5% FCM, kg/d	26.9	1.25	22.6	1.28	24.4	1.22	25.0	1.26	0.02	0.74	0.47
Milk fat, %	4.41	0.1408	4.18	0.1434	4.25	0.1373	4.34	0.1415	0.26	0.67	0.26
Milk protein, %	3.48	0.0490	3.38	0.0499	3.49	0.0478	3.37	0.0492	0.15	0.09	0.26
Milk lactose, %	4.88	0.0336	4.79	0.0342	4.86	0.0328	4.81	0.0338	0.07	0.22	0.67
Milk SCC, $\log_{10}$	4.88	0.1231	4.97	0.1253	4.94	0.1199	4.92	0.1236	0.62	0.90	0.08

[1]Diets contained 0.2 to 0.4% Ca (NC) or 1.2 to 1.5% Ca (HC) and were fed from dry-off until the subsequent calving. Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Probability values are presented for the main effects of bST treatment, diets, and the interaction of bST treatment and diet.

statistically elevated postpeak cumulative milk production (35). In their study (35), the heifers treated with bST had higher SCC and a higher incidence of mastitis. In the present Jersey study and in our follow-up trial with Holsteins, milk SCC were unaffected by treatment. Ewes treated with bST during late gestation had greater milk production, and there was no effect on fetal or neonatal development (36).

Neither feed DM nor NE_L intake was affected by bST or diet either prepartum or during the first 9 wk postpartum. Although the number of days Jersey cows were off feed prior to calving was increased for cows fed the HC diets, this effect might have been related to the increased incidence of milk fever and downer cows syndrome in that group. The increased incidence of diarrhea in cows treated with bST prepartum was in agreement with previous observations for lactating cows treated with bST (13). Typically, occurrence of diarrhea in cows treated with bST has been coincident with increased feed intake (13), which did not occur in this study. To date, the observation of increased secondary ketosis with bST treatment has been limited to the disease model for Jersey cows. The explanation for this response is uncertain. Maisey et al. (25) did not observe an effect of prepartum treatment with bST on blood BHBA concentrations or on incidence of ketosis. Treatment with bST did not affect incidence of ketosis in the study of Law et al. (22). In addition, bST treatment during established lactation did not affect either blood ketone concentrations or the incidence of ketosis (4, 6, 10,

TABLE 7. Effect of a normal (NC) or high Ca (HC) diet[1] and treatment with bST (none or 500 mg/14 d) administered to periparturient Jersey cows on feed intake.

	Treatment								P		
	bST				Diet						
Variable	0 mg		500 mg		NC		HC		bST	Diet	bST × Diet
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE			
DMI, kg/d											
Prepartum	9.4	0.50	8.5	0.54	8.5	0.52	9.4	0.52	0.22	0.20	0.29
Postpartum	13.4	0.56	13.3	0.60	14.0	0.58	12.7	0.58	0.93	0.13	0.59
NE_L Intake, Mcal/d											
Prepartum	15.0	0.83	13.5	0.89	13.0	0.86	15.4	0.86	0.24	0.06	0.32
Postpartum	22.9	0.97	22.7	1.03	24.2	1.02	21.4	1.02	0.93	0.06	0.54
NE_L Balance, Mcal/d											
Postpartum	1.27	0.51	2.29	0.72	2.47	0.51	1.09	0.53	0.17	0.07	0.35
Ca Intake, g/d											
Prepartum	95	6.0	93	6.3	35	6.1	153	6.2	0.78	<0.01	0.91
Postpartum	122	5.2	117	5.6	146	11.2	94	11.2	0.54	0.01	0.23

[1]Diets contained 0.2 to 0.4% Ca (NC) or 1.2 to 1.5% Ca (HC) and were fed from dry-off until the subsequent calving. Subcutaneous injections of excipient or 500 mg of bST in excipient were given every 14 d from 28 d prior to expected calving until approximately 14 d postcalving. Probability values are presented for the main effects of bST treatment, diets, and the interaction of bST treatment and diet.

26). Further work may be warranted to understand response to bST during the periparturient period.

CONCLUSIONS

Treatment of mature Jersey cows with bST during the periparturient period did not affect the incidence of milk fever, decreased the incidence of clinical mastitis (in the HC group only), and increased the duration, but not the incidence, of ketosis compared with those of control cows. Data for blood analytes confirmed the clinical responses but also indicated that cows treated with bST mobilized more bone Ca. The effect on mastitis was detected only for the treated cows that were fed the HC diet and must be considered as preliminary given the small size of the study. The HC diets increased the incidence of milk fever and downer cow syndrome compared with the incidence in cows fed the NC diets. Treatment with bST during the periparturient period was associated with decreased 3.5% FCM production in this test but not in the follow-up study. Neither bST treatment nor HC diets for dry cows affected the incidence of health disorders of the circulatory-lymphatic, genito-urinary, respiratory, optical, or integumentary systems or the incidence of elevated body temperature or allergic reaction. The potential for bST as a prophylactic treatment remains, but additional studies are needed to confirm health effects.

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